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Glutathione S-transferase in marine invertebrates from Langesundfjord

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ABSTRACT: Glutathione S-transferase (GST), an enzyme system which conjugates glutathione to a variety of xenobiotics with electrophilic centres, was found during the GEEP Workshop in the digestive glands of the snail *Littorina littorea* and the mussel *Mytilus edulis*, and in the hepatopancreas of the crab *Carcinus maenas*. GST activity was significantly higher in crabs from 2 polluted sites in Langesundfjord, Norway, relative to reference-site crabs but no such differences were found for mussels, in spite of large differences in PAH and PCB tissue concentrations between the sites. In a mesocosm experiment involving diesel oil and copper dosing, no significant effects were observed on crab and mussel GST activity though *L. littorea* showed significantly higher GST activity at the highest contaminant dose. Since few studies have been done on GST induction in marine invertebrates, it is not clear how differences in GST activity in crabs from the field sites can be related to the pollutants present.

INTRODUCTION

Glutathione S-transferase (GST) catalyses the conjugation of glutathione with xenobiotic compounds containing electrophilic centres. Arene oxides produced by the action of cytochrome P-450 systems on aromatic compounds can be conjugated to glutathione by GST. It is important for organisms to deal with active electrophiles since they can react with macromolecules controlling cell growth such as DNA, RNA and proteins. Many, if not all, chemical carcinogens are electrophiles (Miller & Miller 1979). Thus GST plays an important role in detoxifying strong electrophiles having toxic, mutagenic and carcinogenic properties. Toxic electrophiles found in marine waters include a variety of xenobiotics, such as the organophosphorus insecticides. GST occurs in cytosol of tissues in a number of marine invertebrates (Tate & Herf 1978, James et al. 1979, Balabaskaran et al. 1986).

Vertebrate livers have a large number of GST isozymes (Reddy et al. 1983, Miyaura & Isono 1986, Ramage et al. 1986). Invertebrates examined to date, primarily insects, have shown only 1 or 2 isozymes (Motoyama & Dauterman 1977, Yawetz & Agosin 1981, Clark & Shamaan 1984, Yawetz & Koren 1984). Recently we have purified GST isozymes from the hepatopancreas of the blue crab *Callinectes sapidus* (Keeran & Lee 1987). Two isozymes were found which differed with respect to isoelectric points, subunit composition and molecular weight. The crab hepatopancreas has been shown through both in vivo and in vitro studies to play a major role in metabolism of xenobiotics. Different cell types found in hepatopancreas tubules include E-, F-, R- and B- cells. The F-, R- and B-cells are derived from embryonic or E-cells. The R-cells are storage cells while the F- and B-cells are thought to be important in protein synthesis (Johnson 1980).

A wide range of xenobiotics are inducers of cytosolic GST activity in mammals. These include mixed-function oxygenase inducers, such as phenobarbital, polycyclic aromatic hydrocarbons (PAH) and polychlorinated biphenyls (PCB), as well as phenolic anti-oxidants (Hales & Neims 1977, Lu 1979, Pearson et al. 1983, Schramm et al. 1985). Anti-oxidants are the most potent inducers with mouse GST activity elevated 11-fold by administration of 2(3)-tertiary-butyl-4-hydroxyanisole (Pearson et al. 1983). Few studies have been done on the induction of GST activity in aquatic invertebrates. Recent experiments in our laboratory revealed that addition of the anti-oxidant, 2,6-ditertiary-butyl-4-hydroxytoluene, to the food resulted in a 2-fold increase in hepatopancreas GST activity in crabs *Callinectes sapidus*, and shrimp *Penaeus setiferus* (Keeran & Lee unpubl.).

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Table 1. Glutathione S-transferase activity (GST, nmol min^{-1} mg $protein^{-1}$) in *Mytilus edulis (M. e.)* digestive gland and *Carcinus maenas (C. m.)* hepatopancreas cystosol, at the 4 Langesundfjord sites (means ± SD, $n = 3$). Also, tissue concentrations (ng g^{-1} dry wt) of polycyclic aromatic hydrocarbons (PAH) and polychlorinated biphenyls (PCB), from Appendix 1

	GST		PAH		PCB	
Site	*C. m.*	*M. e.*	*C. m.*	*M. e.*	*C. m.*	*M. e.*
1	254 ± 30	78 ± 12	75	2200	160	75
2	263 ± 42	59 ± 22	115	5900	320	180
3	388 ± 25••	87 ± 10	180	11 500	340	225
4	427 ± 52••	37 ± 20	245	15 500	480	275

Significant difference from reference site (1) indicated by •• $p < 0.01$

Table 2. Glutathione S-transferase activity (nmol min^{-1} mg $protein^{-1}$) in digestive gland (*Mytilus edulis* and *Littorina littorea*) and hepatopancreas (*Carcinus maenas*) cytosol, in the 4 experimental mesocosm basins (means ± SD, $n = 3$)

Basin	*C. maenas*	*M. edulis*	*L. littorea*
C	368 ± 51	53 ± 10	748 ± 54
L	238 ± 14	74 ± 13	697 ± 21
M	344 ± 29	64 ± 26	678 ± 89
H	325 ± 98	72 ± 8	868 ± 70•

Significant difference from control (C) indicated by • $p < 0.05$

The focus of the present study was to determine GST activity in hepatopancreas or digestive gland of mussels *Mytilus edulis*, snails *Littorina littorea* and crabs *Carcinus maenas* both from a contaminant dosing experiment in the Solbergstrand mesocosms, and from 4 sites on a pollution gradient in Langesundfjord, Norway (mussels and crabs only). Specifically, we have examined the possibility that GST activity could be used as an early warning of pollution problems in the field.

MATERIAL AND METHODS

Field sites and sample collection are described by Follum & Moe (1988), and Bakke et al. (1988) explain the dosing regime for the mesocosm experiment (a diesel oil and copper mixture at 4 concentrations; C: control, L: low, M: medium and H: high dose).

Hepatopancreas or digestive glands of *Mytilus edulis, Littorina littorea* and *Carcinus maenas* were dissected and homogenised in 0.25 *M* Tris buffer (pH 8.0). To protect the transferase against protease activity the buffer contained phenylmethylsulphonyl fluoride (PMSF: 0.5 *M*) and trypsin inhibitor (1 g l^{-1}). The homogenate was centrifuged twice at 12 000 × *g* for 10 min and the supernatant was centrifuged at 100 000 × *g* for 60 min. The 100 000 × *g* supernatant, i.e. cytosol, was used for all assays.

The standard assay for glutathione S-transferase activity used 1-chloro-2,4-dinitrobenzene as the substrate and followed the methods described by Habig et al. (1974). Specific activity was expressed as moles of substrate converted per min per mg of protein. Procedures described by Ahearn et al. (1983) were used to prepare B-, F- and R-cells from crab hepatopancreas. Collagenase was used to induce tissue dissociation which was followed by separation of cell types in a density gradient.

RESULTS

The hepatopancreas of *Carcinus maenas* and the digestive glands of *Mytilus edulis* and *Littorina littorea* all exhibited GST activity (Tables 1 and 2), the highest being in *L. littorea*, followed by *C. maenas* and *M. edulis* (respectively 680 to 870, 240 to 430 and 40 to 90 nmol min^{-1} mg $protein^{-1}$). Activity differed among the F-, B- and R-cell types isolated from crab hepatopancreas (respectively 1100 to 1500, 80 to 160 and 30 to 50 nmol min^{-1} mg $protein^{-1}$).

In the field samples no significant differences were observed in GST activity in mussel digestive gland in spite of a clear gradient in tissue concentrations of PAH and PCB (Table 1). However, GST activity was significantly higher in *Carcinus maenas* hepatopancreas from Sites 3 and 4 compared with Sites 1 and 2 (Table 1), as shown by a 1-way analysis of variance (Sokal & Rohlf 1969). This corresponded well with an increasing gradient of PAH concentrations in crab tissue across Sites 1 to 4, and a higher concentration of PCB at Sites 2 to 4 relative to the reference site.

In the mesocosm samples there was no significant difference in GST activity in mussels or crabs from the different treatments (Table 2) but activity in *Littorina littorea* from the high dose was significantly higher ($p < 0.05$) than that from the other 3 basins.

DISCUSSION

GST activity was found in cytosol from digestive glands of *Littorina littorea* and *Mytilus edulis* and the hepatopancreas of *Carcinus maenas*. The high activity in the F-cells of *C. maenas* hepatopancreas was not surprising, since these cells have extensive endoplasmic reticulum and Golgi network which is characteristic of cells involved in protein synthesis. The digestive gland of *L. littorea* is composed of 2 principal cell types, digestive cells and excretory or basophil cells (Merdsoy & Farley 1973, Mason et al. 1984, Pipe 1986, Pipe & Moore 1986). No attempt was made to separate these 2 cell types. The basophil cells have a well-developed system of rough endoplasmic reticulum and Golgi network (Mason et al. 1984). We speculate that basophil cells have functions similar to those of F-cells in crabs and would have high GST activity.

The high GST activity in *Littorina littorea*, compared with *Carcinus maenas* and *Mytilus edulis* was of interest. We suggest that the high GST activity in *L. littorea* was due to secondary metabolites found in their diet of macroalgae. A number of macroalgae species are known to contain high concentrations of secondary metabolites, often unsaturated aldehydes, which have cytotoxic properties and electrophilic centres (Fenical 1982). Recent studies in our laboratory have shown very high GST activity in herbivorous gastropods, including *L. littorina*, from tropical waters. Feeding these gastropods on algae with high concentrations of unsaturated aldehydes resulted in an increase in the digestive gland GST activity (Lee unpubl.).

As noted earlier there have been few studies of the effects of pollutants on GST activity in marine animals. Administration of a PAH-like inducer, ß-naphthoflavone, resulted in a 2-fold increase in GST activity in rainbow trout liver (Andersson et al. 1985). Among aquatic invertebrates, pentachlorophenol and butylated hydroxytoluene have been shown to increase GST activity in fresh water cladocerans and marine crustaceans, respectively (LeBlanc & Cochrane 1985, Lee unpubl.). We are not aware of any published work showing an increase in GST activity in marine invertebrates after exposure to PAH or PCB. Thus, the cause of the elevated GST activity in crabs from Sites 3 and 4 relative to Sites 1 and 2 is not known. Besides the PAH and PCB found in the tissues, it is likely that other classes of organic pollutants which could act as GST inducers in crabs were present. For example, octachlorostyrene and hexachlorobenzene were found in fish collected from the same Langesundfjord sites (Addison & Edwards 1988).

No significant differences in GST activity were noted in *Mytilus edulis* from the field sites, in spite of differing tissue concentrations of PAH and PCB. In other studies, GST activity was low in all filtering bivalve molluscs that were assayed (Lee unpubl.). Suteau et al. (1988) report their work on GST activity in *M. edulis*. They worked with the whole mussel tissue (we used only digestive gland) and ^{3}H-styrene oxide as substrate for GST (we use chlorodinitrobenzene as substrate). No significant differences were noted in GST activity in *M. edulis* from the different mesocosm treatments. They found mussels from Field site 2 to have significantly higher GST activity than mussels from the other sites. We found no significant differences between Site 2 and the reference site, for either mussels or crabs. The radiometric method using ^{3}H-styrene oxide is a far more sensitive GST assay than the spectrophotometric assay we used; our GST activity for mussels reported in Table 1 is only slightly above the detectable level. In addition, various GST isoenzymes have different activities with different substrates. Thus, there may not be a conflict between our results and those of Suteau et al. (1988).

In the mesocosm experiment, with the exception of *Littorina littorea* in the highest dose basin, there were no significant differences in GST activity between different diesel oil and copper concentrations, for all 3 species. Diesel oil has only low concentrations of mixed function oxygenase and GST inducers, i.e. higher weight PAH, and the effect of copper on GST activity is unknown. We speculate that oxidised products of diesel oil, e.g. aldehydes and ketones, could have acted as GST inducers in *L. littorea*.

While GST activity was significantly higher in the hepatopancreas of crabs from 2 of the polluted field sites, relative to the reference site, much work is required before it can be determined if this assay is useful for field studies of organic pollution. Laboratory studies have shown that GST activity in the hepatopancreas of marine crustaceans increase 2-fold when certain anti-oxidants are present in their food (Lee unpubl.). In snails, certain algae metabolites, possibly unsaturated aldehydes, can increase GST activity (Lee unpubl.). If new GST isozymes are produced in response to certain classes of pollutants then assays for these induced isozymes, e.g. antibody reactions, would probably be a useful way to monitor for the effects of certain active electrophiles in marine waters.

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